SYNTHESIS OF [1',3',4'-$^3H_3$]4-(5',6',7',8'-TETRAHYDRO-5',5',8',8'-TETRAMETHYL-2'-ANTHRACENYL)BENZOIC ACID FOR BINDING STUDIES OF RETINOIC ACID RECEPTORS

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SUMMARY

[1',3',4'-$^3H_3$]4-(5',6',7',8'-Tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic acid (specific activity 64 Ci/mmole) was synthesized by bromination of ethyl 4-(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoate, followed by reductive debromination by tritium gas in the presence of 10% palladium on carbon and hydrolysis. ^1H and ^3H NMR spectroscopy was used to establish tritium at 1',3',4'-tetrahydroanthracenyl ring positions.

Keywords. RAR, retinoic acid receptor, retinoid, [1',3',4'-$^3H_3$]4-(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic acid, [$^3H_3$]TTAB.

INTRODUCTION

The discovery that all-*trans*-retinoic acid exerts its biological effects by binding to and activating the retinoic acid receptors (RAR-α, RAR-β, and RAR-γ), which regulate gene transcription by binding to specific DNA response elements (1-6), has stimulated the search for receptor-specific retinoids for mechanistic studies and as potential therapeutic agents. Although tritiated retinoic acid has been used for these receptor binding studies, its susceptibility to oxidative and photolytic degradation makes its use impractical on an extended basis. We (7) and others (8) have synthesized 4-(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic acid (TTAB), which has high affinity for all three retinoic acid receptor proteins in competitive binding experiments with [11,12-$^3H_2$]all-*trans*-retinoic acid (Dawson *et al.*, in press) that is comparable to that of all-*trans*-retinoic acid. Nonspecifically tritiated TTAB (specific activity 52 Ci/mmol) that was prepared by a noncatalyzed bromination of TTAB ethyl ester with 2.2 equiv. of Br_2, followed by hydrogenolysis and ester hydrolysis was claimed in a patent that contained no characterization information (9). To provide a procedure for others involved in retinoid receptor studies we report here a facile synthesis of a specifically tritritiated TTAB, involving bromination and palladium-catalyzed tritium exchange.

0362–4803/93/070633–05$07.50

Received 17 February, 1993
Revised 15 March, 1993

RESULTS AND DISCUSSION

The ethyl ester of TTAB (**1**) brominated rapidly at room temperature in the presence of iron powder as a catalyst. Three equivalents of bromine produced a mixture consisting predominantly of ethyl 4-(1',4'-dibromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoate (**2**) and ethyl 4-(1',3',4'-tribromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoate (**3**), which were separated by tedious fractional crystallizations from MeCN (Figure 1).

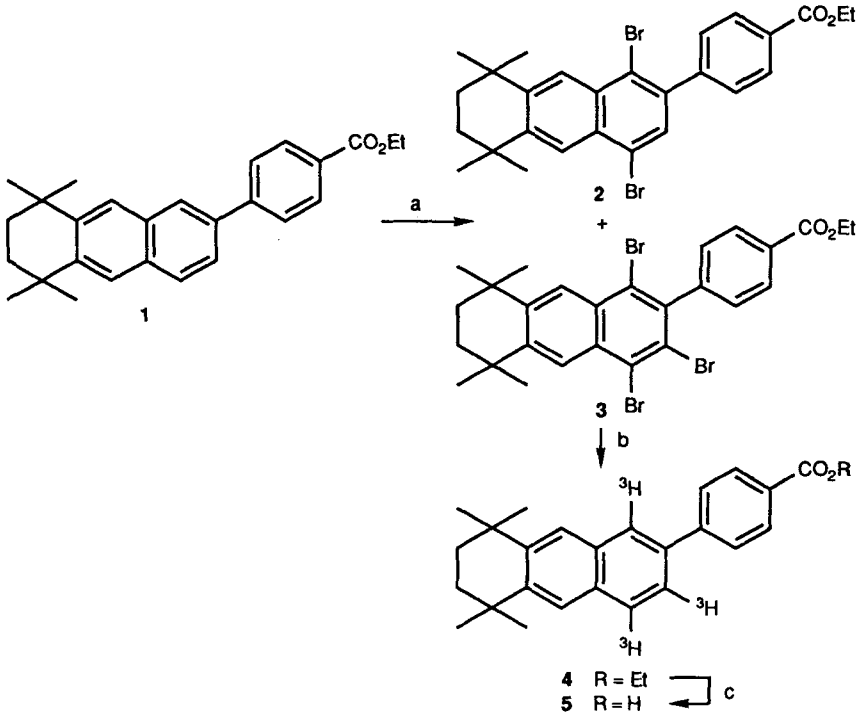

Figure 1. Synthesis of [$^3H_3$]TTAB (**5**): a. Br_2, Fe, CH_2Cl_2. b. $^3H_2$, 10% Pd(C), Et_3N, EtOAc. c. 40% aq. KOH in EtOH; aq. HCl.

Excess bromine afforded after 1.5 h at room temperature the 1,3,4-tribromotetrahydroanthracene **3** in 46% yield. The bromination product was uncontaminated by the 1,4-dibromotetrahydroanthracene **2**. The byproducts were largely polar compounds, originating from Lewis acid ($FeBr_3$) or acid (HBr) catalyzed decomposition. Prolonged reaction times with either iron powder or $TiCl_4$ as the catalyst gave only decomposition products.

The ethyl ester **3** of 1',3',4'-Br_3-TTAB was deuterated first to establish labeling specificity. After hydrolysis, [1',3',4'-$^2H_3$]TTAB was isolated in 73% yield. The absence of the 1', 3', and 4'-proton signals in the ^1H NMR spectrum established the sites of deuterium incorporation. The tritiation was conducted similarly. [$^3H_3$]TTAB having a specific activity

of 64 Ci/mmol was characterized by HPLC to have a chemical purity equal to or greater than 99.9% and a radiochemical purity of 96.9%. ^3H NMR showed ^3H substitution at the 1', 3', and 4'-positions of the tetrahydroanthracene ring.

EXPERIMENTAL

The following instruments were used for characterization: melting points (Thomas Hoover Unimelt capillary melting point apparatus, uncorrected); IR spectra (Perkin-Elmer 1600 FTIR spectrophotometer); UV spectra (unlabeled compounds: Perkin-Elmer Lambda 2 spectrophotometer; labeled compounds: Hewlett-Packard 104A diode array detector coupled to HPLC); NMR spectra (unlabeled ^1H: Varian XL 400 and Gemini 300 spectrometers; labeled ^1H and ^3H: Bruker NR/300 spectrometer); HPLC (labeled: Waters Associates 510 pump, equipped with a Hewlett-Packard 104A diode array UV detector, Berthold ^3H detector, and Tracor display). Liquid scintillation counting was performed on a Packard 1500 Tri-Carb instrument.

Ethyl 4-(1',3',4'-Tribromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoate (3). To a solution of 138 mg (0.357 mmol) of ethyl 4-(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoate (**1**) [7] and 0.45 g (2.8 mmol) of Br_2 in 3 mL of CH_2Cl_2 was added approximately 20 mg (0.35 g-atom) of Fe powder. The red suspension was stirred in the dark at room temperature with water-bath cooling and protection from moisture ($CaSO_4$) for a period of 1.5 h. The reaction mixture was then diluted with CH_2Cl_2 (20 mL) and washed with H_2O (2 × 10 mL), aq. $Na_2S_2O_3$ (2 × 10 mL), and H_2O (10 mL), dried by filtration through a pad of Na_2SO_4, and concentrated. The orange residue was chromatographed twice on 1.5-cm × 25-cm silica gel columns (3 CH_2Cl_2: 7 hexanes; then 5 Et_2O: 95 hexanes) to give 102 mg (46%) of the ester **3** as pale-yellow needles, m.p. 180-180.5°C (MeCN); TLC (silica gel, 5 EtOAc: 95 hexanes) R_f 0.56; IR ($CHCl_3$) 3010, 2950, 1710, 1610, 1470, 1360, 1270, 1190, 1110, 1015, 920, 880, 840 cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$) δ 1.42 (t, J = 7 Hz, 3 H, $CH_2C\underline{H}_3$), 1.43 and 1.45 (2 s, 12 H, 5',8'-CMe_2), 1.81 (m, 4 H, 6',7'-$(CH_2)_2$), 4.42 (q, J = 7 Hz, 2 H, OCH_2), 7.29 (d, J = 9 Hz, 2 H, 3,5-ArH), 8.18 (d, J = 9 Hz, 2 H, 2,6-ArH), 8.28 and 8.32 (2 s, 2 H, 9',10'-ArH); UV (EtOH) λ_{max} 309 nm (ε 6.7 × 10^3), 248 nm (ε 7.0 × 10^4); HR EIMS, M = 619.9 (M^+, $C_{27}H_{27}{}^{79}Br_3O_2$).

Ethyl 4-(1',4',-Dibromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoate (2). Bromination of 170 mg (0.44 mmol) of ethyl ester **1** with 0.30 g (1.9 mmol) of Br_2 and 20 mg (0.35 g-atom) of Fe powder in 4 mL of CH_2Cl_2, followed by the work-up and chromatographic conditions used for the preparation of **3**, gave 156 mg of a mixture of products from which 52 mg (22%) of the 1,4-dibromotetrahydroanthracene **2** was isolated as dense, white crystals, m.p. 140.5-141°C by crystallization from MeCN (4 × 3 mL): 400 MHz ^1H NMR ($CDCl_3$) δ 1.43 (t, J = 7 Hz, 3 H, $CH_2C\underline{H}_3$), 1.45 and 1.46 (2 s, 12 H, 5',8'-CMe_2), 1.82 (m, 4 H, 6',7'-$(CH_2)_2$), 4.43 (q, J = 7 Hz, 2 H, OCH_2), 7.50 (d, J = 9 Hz, 2 H, 3,5-ArH), 7.62 (s, 1 H, 3'-ArH), 8.15 (d, J = 9 Hz, 2 H, 2,6-ArH), 8.21 (2 s, 2 H, 9',10'-ArH); EI-MS, M = 542 (M^+, $C_{27}H_{28}{}^{79}Br_2O_2$).

[1',3',4'-$^2H_3$]4-(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic Acid. A solution of 16 mg (26 μmol) of tribromo ester **3** in 3 mL of EtOAc was deuterated (99.9% $^2H_2$, Matheson) in the presence of 17 mg of 10% Pd(C) (Alfa) at room temperature for 3 h. The residue obtained after filtration (Celite) and concentration was heated at reflux for 2.5 h with 3 mL of 40% ethanolic KOH. Acidification (3 mL of 6 N HCl), Et_2O extraction (5 × 10 mL), brine washing (2 × 10 mL), drying ($MgSO_4$), and concentration afforded 6.7 mg (73%) of the deuterated acid as a pale-yellow solid: HPLC (8-mm × 10-cm Novapak C_{18}, 95 MeCN: 4.9 H_2O: 0.1 TFA, 276.4 nm, 1.5 mL/min) t_R 4.56 min; 300 MHz ^1H NMR ($CDCl_3$) δ 1.42 (2 s, 12 H, 5',8'-CMe_2), 1.79 (m, 4 H, 6',7'-$(CH_2)_2$), 7.81 (d, J = 8 Hz, 2 H, 3,5-ArH), 7.83 and 7.87 (2 s, 2 H, 9',10'-ArH), 8.19 (d, J = 8 Hz, 2 H, 2,6-ArH); HR EIMS, M = 361.2 (M^+, $C_{25}H_{23}D_3O_2$).

[1',3',4'-$^3H_3$]4-(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic Acid (5). A solution of 16.0 mg (25.5 μmol) of ethyl 4-(1',3',4'-tribromo-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoate (**3**) and 50 μL (360 μmol) of Et_3N in 3 mL of EtOAc containing 28 mg of dried (100°C) 10% Pd(C) (Alfa) was degassed by three liquid N_2 freeze-and-thaw cycles. The degassed suspension was stirred under a $^3H_2$ atmosphere at room temperature for 3 h. Residual $^3H_2$ was removed, and the reaction mixture was concentrated at reduced pressure. The residue was treated with MeOH (2 × 1 mL) for $^3H_2$ back-exchange and reconcentrated at reduced pressure. This residue was extracted with EtOAc (2 × 4 mL) and filtered (glass-fiber disk). The filtrate was lyophilized to dryness to give ethyl [1',3',4'-$^3H_3$]4-(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoate (**4**) as a pale-yellow solid. Ester **4** dissolved in 3 mL of EtOH was heated at reflux with 1 mL of 40% aqueous KOH (5.6 mmol) for 1 h and stirred at ambient temperature overnight. The resulting solution was cooled in an ice-bath and acidified by the dropwise addition (10 min) of 3 mL of 6 N HCl. The mixture was diluted with H_2O (5 mL) and extracted with Et_2O (5 × 10 mL). The combined extracts were washed with saturated brine (2 × 10 mL), dried ($MgSO_4$), filtered (sintered glass frit, 5 × 2 mL of Et_2O rinse), and concentrated under a stream of N_2 to give 8.95 mg (97%) or 1.6 Ci of **5** as a pale-yellow solid, which was dissolved in 2.5 mL of EtOAc. The chemical purity of **5** was determined to be greater than 99.9% by HPLC (t_R 4.56 min with no other UV peaks observed), which also indicated a radiochemical purity of 96.9% [t_R 3.26 (2.6%), 4.56 (96.9%), and 6.21 min (0.4%)]. UV (95 MeCN: 4.9 H_2O: 0.1 TFA) λ_{max} [$^3H_3$]TTAB: 226 (0.93), 2.76 (1.0), 314 nm (0.51 relative absorbance); UV TTAB λ_{max} 226 (0.92), 276 (1.0), 314 nm (0.51). ^3H NMR ($C_6{}^2H_6$) δ 7.49 (d, J = 9 Hz, 1 ^3H, 3'-Ar-^3H), 7.71 (d, J = 9 Hz, 1 ^3H, 4'-Ar-^3H), 7.82 (s, 1 ^3H, 1'-Ar-^3H).

Acknowledgement. Support of this work by USPHS Grants CA51993 (M.I.D.) and RR01237-10 is gratefully acknowledged.

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